DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date JUN 24 1997

From Acting Director, Division of Programs and Enforcement Policy, Office of Special Nutritionals, HFS-455

Subject 75-Day Premarket Notification for New Dietary Ingredients

To Dockets Management Branch, ~~HFS~~ HFA-305

97 7338 '97 JUL -3 A9 :20

New Dietary Ingredient: 7-keto DHEA acetate

Firm: General Nutrition Corporation
Date Received by FDA: May 27, 1997
90-Day Date: August 25, 1997

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after August 25, 1997.

Sincerely yours,

James Tanner

James Tanner, Ph.D.
Acting Director,
Division of Programs and
Enforcement Policy
Office of Special Nutritionals
Center for Food Safety and
Applied Nutrition

Attachment

cc:
HFS-22, CCO
HFS-450 (r/f, OSN w/control slip:TRAC#52736 & cpy incoming)
HFS-456 (r/f, Latham, Moore)
r/d:HFS-456:JELatham:jel:06/13/97:DocName:#52736.mem:Disc3

95S-0316

RPT 14

GNC
GENERAL NUTRITION CENTERS
LIVE WELL

CONFIDENTIAL

General Nutrition Corporation
921 Penn Avenue
Pittsburgh, PA 15222
412/288-4600

May 14, 1997

Linda S. Kahl, Ph.D.
Office of Special Nutritionals
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W. (HFS-450)
Washington, DC 20204

Dear Dr. Kahl:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, General Nutrition Corporation ("GNC"), on its own behalf and on behalf of Humanetics Corporation, St. Louis Park, Minnesota, wishes to notify the Food and Drug Administration that it will market a new dietary ingredient, 7-keto DHEA acetate, the acetylized form of 7-keto DHEA, a metabolite of DHEA, a dietary ingredient on the market prior to October 15, 1994. Accordingly, enclosed please find two (2) copies of this notification.

The dietary supplement which contains 7-keto DHEA acetate will consist of twenty-five (25) mg of 7-keto DHEA acetate in a tablet or capsule which will be suggested to be taken up to two times per day.

Attached please find a summary and reports of the safety studies and other information which establish that this dietary ingredient, when used under the conditions suggested in the labeling of the dietary supplement, is reasonably expected to be safe. These supporting studies include:

1. Three page safety profile summary of 7-keto DHEA acetate with references to published literature.

2. Escalating Dose Oval Gavage Toxicity Study with 7-keto DHEA Acetate in Rhesus Monkeys. Abstract of Report by Convance Laboratories, Inc. (formerly Corning Hazelton Laboratories).

3. Acute Oral Gavage Toxicity Study with 7-keto DHEA Acetate in Rats. Abstract of Report by Convance Laboratories, Inc.

4. Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with 7-keto DHEA Acetate. Abstract of Report by Convance Laboratories, Inc.

5. Lack of Toxicity of 7-keto DHEA in Rhesus Monkeys. Abstract of Report by Kemnitz, et al.

6. Assorted reprints of references.

The full reports of items (2) - (5) are available for review upon request and so long as the confidentiality of the full reports, which constitute confidential commercial information, can be maintained.

Very truly yours,

John P. Troup

John P. Troup, Ph.D.
Vice President
Scientific Affairs

JPT/jaj

cc: Mr. Ronald J. Zenk
President
Humanetics Corporation

7-keto DHEA Acetate

Basis for Concluding New Dietary Ingredient Will Reasonably Be Expected to be Safe

Background

Dehydroepiandrosterone (DHEA) is a dietary ingredient which was marketed in the United States before October 15, 1994. 7-oxo-DHEA (7-keto DHEA) is a well-known metabolite of DHEA in humans. The occurrence of 7-keto-DHEA in urine of healthy and diseased persons was first reported by Lieberman et al. in 1948. They described the properties of the compound, but were unable to assign a specific structure.

In 1954, Fukushima et al. reported that 7-keto-DHEA is found in urines from both normal and abnormal subjects; it is excreted in larger amounts in adrenal abnormalities, especially those characterized by increased excretion of dehydroepiandrosterone (DHEA). Gallagher (1958) found that amphenone, an inhibitor of steroid hormone synthesis, suppressed the urinary excretion of cortisol and dehydroepiandrosterone, but had relatively little effect on the excretion of 7-keto-DHEA. Baulieu et al. 1961 showed that 7-keto-DHEA is present as the sulfate (at the 3-position) as is DHEA. Levels of 7-keto-DHEA sulfate in the blood from six patients ranged up to 40% of those of DHEA sulfate. Faredin et al. (1969) found that enzymes in human skin *in vitro* metabolized DHEA to 7-oxygenated derivatives, including 7-keto-DHEA. 7-Alpha-hydroxy-DHEA was detected in human blood and tissues (Parker, 1989), as a result of hydroxylase enzyme acting on DHEA; 7-keto-DHEA was also present.

7-keto-DHEA was first synthesized and identified by Billeter and Miescher also in 1948. 3-Beta-acetoxy-androst-5-en-7, 17 dione (7-keto-DHEA acetate) is an acetylated form of 7-keto-DHEA. The acetate group is added to protect the 3 position from oxidation that occurs during the manufacturing of 7-keto-DHEA acetate. 7-keto-DHEA acetate is readily converted to 7-keto-DHEA by esterases located in the blood and in body tissues. Similar compounds (i.e., DHEA) are manufactured and consumed as acetate esters.

Safety Assessments

Several safety assessments were performed using 7-keto-DHEA acetate or 7-keto-DHEA. The mutagenic activity of 7-keto-DHEA acetate and/or its metabolites was assessed for their ability to induce reverse mutations at the histidine locus in the genome of specific Salmonella tyhimurium tester strains and at the tryptophan locus in an Escheichia coli tester strain both in the presence and absence of exogenous metabolic activation system of mammalian microsomal enzymes (S-9) derived from Aroclor-induced rat liver. Doses of 7-keto-DHEA acetate ranged from 0.1 to 5.0 mg per plate. 7-keto-DHEA acetate did not cause a positive increase in the number of revertants per plate in any of the tester strains either in the presence or absence of S-9.

The acute toxicity of 7-keto-DHEA acetate, administered as single oral gavage doses, was assessed in five groups of Crl: CD (SD) BR VAF/Plus rats (five/sex/group) at dose levels of 0, 250, 500, 1000 or 2000 mg/kg. All animals survived to the scheduled day 15 sacrifice. There were no differences in body weight or body weight gain attributed to test material. 7-keto-DHEA acetate had no apparent effect on anatomical pathology results. Thus, the no-observable-adverse-effect level for 7-keto-DHEA acetate was greater than 2,000 mg/kg.

Safety was also addressed in rhesus monkeys in two separate studies. In one study, four monkeys (2/sex) received 7-keto-DHEA acetate by oral gavage at dose levels of 250, 500 and 1000 mg/kg on days 1, 3 and 5 respectively and 1000 mg/kg on days 7 through 11. Blood samples were collected for hematology and clinical chemistry tests on day 0, 7 and 12. Animals were anaesthetized, exsanguinated and necropsied on day 12. Administration of 7-keto-DHEA acetate at a level of 1000 mg/kg for five days had no apparent effects on clinical or anatomical pathology results.

In another rhesus monkey study, a total of 16 male monkeys, from 4 to 8 years old, were used in two experimental groups (Kemnitz et al., 1997). First, 7-keto-DHEA was fed to groups of 2, 2 and 3 monkeys at doses of 7, 35 and 70 mg/kg daily, respectively. 7 and 35 mg/kg animals received compounds for one week while the high dose group received compound for two weeks. Liver biopsies

were obtained prior to dosing and on days 7 (or 14) and 28. In the additional experimental group, dosing was extended to 28 days. Groups of three monkeys received placebo, 25 ug thyroxin or 140 mg/kg 7-keto DHEA. Liver biopsies for histological examinations were taken before and at the end of the dosing period. In both experimental groups, fasting blood samples were drawn weekly. There were no effects on behavior or appearance. 7-keto-DHEA caused no detectable changes in blood chemistries, differential cell count, hemoglobin, hematocrit, mononuclear cell proliferation or liver histology. Body weights were not affected by 7-keto-DHEA.

Unlike DHEA, 7-keto-DHEA is not convertible to active androgens or estrogens. Sunde et al. (1982) reported that 7-alpha-hydroxytestosterone has no androgenic activity as measured by its lack of effect on maintenance of acid phosphatase in castrated rats. Lardy et al. (1995) further demonstrated that 7-hydroxytestosterone does not influence rat seminal vesicle weight nor does it induce thermogenic enzymes. Cedard et al. 1964 found that 7-keto androst-5-ene compounds are not aromatized and thus can not be converted to estrogens.

Dose Considerations

Selection of the dose of 7-keto-DHEA acetate for human consumption was determined from: 1) acute toxicity studies in rats and monkeys; 2) sub chronic exposure in monkeys; and 3) reference to well tolerated doses of DHEA, up to 750 mg TID for 16 weeks in human clinical trials (Dyner et al. 1993), on the rationale that DHEA is readily convertible to 7-keto-DHEA in humans. Based on acute oral safety results in rats (no deaths or untoward clinical signs at doses up to 2000 mg/kg), and rhesus monkey (no deaths, no changes in liver, blood or urine chemistries observed at doses up to 1000 mg/kg) as well the fact that no changes were observed in a 28 day study of 7-keto-DHEA in rhesus monkeys at doses of 140 mg/kg, it was determined that a dose of 25 mg 7-keto-DHEA up to two times daily would be well-tolerated in humans. This dose (50-75 mg/day) is about 1 mg/kg (in a 50-75 kg person) and is 1/2000 or 1/1000 the non lethal dose in rats and monkeys. Also 1 mg/kg 7-keto-DHEA acetate daily is 140-fold lower than the no effect dose given rhesus monkeys for 28 days.

ESCALATING DOSE ORAL GAVAGE TOXICITY STUDY IN RHESUS MONKEYS

Convance Laboratories, Inc.
(formerly Corning Hazelton, Inc.)
3301 Kinsman Boulevard
Madison, Wisconsin 53704

The purpose of the study was to assess the toxicity of the test material, HL-9001 (3 acetyl-7-oxo-DHEA), when administered by oral gavage to rhesus monkeys and to provide a basis for dose selection for subsequent studies.

Four rhesus monkey (two animals/sex) received HL-9001 (3 acetyle-7-oxo-DHEA) by oral gavage at dose levels of 250, 500, and 1,000 mg/kg/day of body weight (mg/kg) on Days 1, 3, and 5, respectively, and 1,000 mg/kg/day on Days 7 through 11. Each group received the dose preparation at a dose volume of 10 mL/kg.

The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. In addition, on each dosing day, animals were observed approximately 1 hour postdose, and at least once daily on nondosing days for abnormalities and signs of toxicity. Body weights were recorded weekly before initiation of treatment, on the first day of treatment, on each dosing day, and on the day of sacrifice. Food consumption was confirmed daily by visual inspection. Blood samples were collected for hematology and clinical chemistry tests before initiation of treatment and predose on Day 7, and on Day 12. On Day 12, fasted animals were weighed, anesthetized, exsanguinated, and necropsied. At necropsy, macroscopic observations were recorded, and selected tissues were collected and preserved.

All animals survived to the scheduled sacrifice on Day 12. There were no test material-related clinical observations when doses were administered at 250 or 500 mg/kg/day; however, at 1,000 mg/kg/day animals vomited postdose. In addition, excessive salivation was observed before, or immediately after dosing on Days 9 through 11. There were no notable effects on body weight. Low or no food consumption was noted for the females on Day 4, and low food consumption was noted for both sexes during the repeated dose phase when animals received 1,000 mg/kg/day.

Oral administration of HL-9001 (3 acetyl-7-oxo-DHEA) to rhesus monkeys at a dose level of 1,000 mg/kg/day for 5 consecutive days had no apparent adverse effects on clinical or anatomical pathology results.

ACUTE ORAL GAVAGE TOXICITY STUDY IN RATS

Convance Laboratories, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

The purpose of this study was to assess the acute toxicity of the test material, HL-9001 (3 acetyl-7-oxo-DHEA), when administered as a single oral gavage dose to rats.

Five groups of Crl:CD®(SD)BR VAF/Plus® rats (five/sex/group) were administered the test material at a dose level of 0 (control group; received carrier, 1% methylcellulose and 0.1% Tween® 80 in reverse osmosis water), 250, 500, 1,000, or 2,000 mg/kg of body weight (mg/kg) at a dose volume of 10 mL/kg.

Food and water were provided *ad libitum*. The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. At least once each week each animal was removed from its cage and examined for abnormalities and signs of toxicity. Body weight data were collected on the day of treatment (Day 1) and weekly thereafter. Food consumption data were collected weekly. After at least 2 weeks posttreatment, animals were fasted overnight, anesthetized with sodium pentrobarbital, weighed, exsanguinated, and necropsied. At necropsy, macroscopic observations were recorded, organ weights were obtained, and tissues were replaced in fixative as specified by the protocol. Microscopic examinations were done on adrenals, brain, heart, kidneys, liver, spleen, and thymus from each animal.

All animals survived to the scheduled sacrifice on Day 15. There were no differences in body weight or body weight gains attributed to the test material. Food consumption for females was significantly higher for all treated groups during Week 2; however, the statistically significant differences were not considered to be toxicologically important.

A single oral administration of HL-9001 (3 acetyl-7-oxo-DHEA) to rats had no apparent effect on anatomical pathology results. The right testis-to-body weight percentages were significantly lower in males give 250 or 1,000 mg/kg; however, in the absence of significant weight differences in the contralateral organ and the absence of correlating macroscopic or microscopic changes, these statistically significant weight changes were considered incidental and unrelated to the test material.

Based on the results of this study, the no-observable-adverse-effect level for a single oral gavage dose of HL-9001 (3 acetyl-7-oxo-DHEA) in male and female Crl:CD®(SD)BR rats was greater than 2,000 mg/kg.

MUTAGENICITY TEST

Covance Laboratories, Inc.
9200 Leesburg Pike
Vienna, VA 22182

At the request of Humanetics Corporation, Covance Laboratories, Inc. investigated HL-9001, 3-acetyl-7-oxo-DHEA, for mutagenic activity in the *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay. This assay evaluated the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of specific *Salmonella typhimurium* tester strains and at the tryptophan locus in an *Escherichia coli* tester strain both in the presence and absence of a exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor™-induced rat liver (S9).

The doses tested in the mutagenicity assay were selected based on the results of a dose range-finding study using tester strains TA100 and WP2*uvr*A and ten doses of test article ranging from 5,000 to 6.67 μg per plate, one plate per dose, both in the presence and absence of S9 mix.

The tester strains used in the mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* tester strain WP2*uvr*A. The assay was conducted with six doses of test article in both the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per dose. The doses tested were 5,000, 2,500, 1,000, 500, 250, and 100 μg per plate in both the presence and absence of S9 mix.

The results of the *Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay indicate that, under the conditions of this study, Humanetics Corporation's test article, HL-9001, 3-acetyl-7-oxo-DHEA, did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor™-induced rate liver (S9).

LACK OF TOXICITY OF 7-KETO-DEHYDROEPIANDROSTERONE IN RHESUS MONKEYS

Joseph W. Kemnitz[1,3], Henry A. Lardy[2], Hideo Uno[1,4], Stefan Gravenstein[3],William B. Ershler[3], E. Gregory MacEwen[5], Ilene Kurzman[5], Wallace D. Houser1, Scott T. Baum[1], Nancy Kneer[2], and Roger Klopp[3]

[1]Wisconsin Regional Primate Research Center, [2]Institute for Enzyme Research, Departments of [3]Medicine and [4]Pathology (School of Medicine) and [5]Medical Sciences (School of Veterinary Medicine), University of Wisconsin, Madison, WI 53715-1299

Subjects and Design

A total of 16 male rhesus monkeys (*Macaca mulatta*) from 4 to 8 years old were used in two experiments as described below. The monkeys were caged individually and fed Purina Monkey Chow (#5037, Ralston Purina Co. St. Louis, MO) supplemented with fresh fruit. Tap water was always available. Room temperature was maintained at approximately 21°C with relative humidity of 50-65%. Lighting was automatically controlled, with lights on from 0600 to 1800 hr. Detailed husbandry procedures have been described (28).

In Experiment 1, 7-keto-DHEA was fed to groups of 2, 2, and 3 monkeys at doses of 7, 35, and 70 mg/kg daily, respectively. The two lower doses were administered for one week and the 70 mg/kg dose was given for two weeks. 7-keto-DHEA was given orally in a piece of fruit. Liver biopsies were obtained by laparotomy before, the day following the last dose, and 28 days later.

In Experiment 2, administration was extended to 28 days. Three monkeys were given placebo, three were fed 25 μg L-thyroxine daily, and three were fed 7-keto-DHEA at 140 mg/kg daily. Liver biopsies for enzyme assays were taken before and at the end of the feeding period.

In both experiments fasting blood samples were drawn weekly during feeding and at the time of the last laparotomy for cell counts and serum chemistry determinations.

Results

Blood Analyses

There were no significant changes in blood hemoglobin, hematocrit, white cell count and white cell differential counts at any dose. All values were within the normal range for healthy Rhesus monkeys. The same holds for blood glucose, BUN, creatinine, protein, SGOT (AST), LDH, GGT and SGPT (ALT).

Total serum cholesterol did not change as a result of feeding in Experiment 1. Also, there were no consistent changes in low- or high-density lipoprotein cholesterol

concentrations. Similarly, there were no detectable effects of feeding on serum cholesterol in Experiment 2.

Immunology

There were no significant changes in mononuclear cell proliferation induced by mitogen treatment nor of natural killer cell activity with any of the feedings. There was a trend toward greater mitogen-induced proliferation in the animals fed with 7-keto-DHEA. For the immunological assays, there is a large interassay variance making measurable significant differences unlikely, especially with such a small sample size.

Liver Histology

Liver sections from the low dose (7mg/kg) group were examined from both animals at day 0 (pretreatment) and 8 and 36 days after initiation of feeding. At day 0 the hepatocytes showed normal cytoplasmic and nuclear configuration and hepatic cell cords arranged in regular radiating patterns. The sinusoid and Glisson's triad showed no abnormalities in blood vessels and bile ducts. The tissues at 8 and 36 days showed essentially no abnormalities: structural features were similar to those of day 0. There were no abnormal structural findings in all sections from two animals on the 35 mg/kg dose. Liver sections were examined from three animals on the 70 mg/kg dose at days 0, 14, and 42. Both hepatocytes and bile ductules in Glisson triads showed no abnormalities at days 0 and 42. At 14 days the hepatocytes showed a mild degree of atrophy, narrowing of the hepatic cell cords, and associated dilation of sinusoidal spaces in two out of three monkeys, but the nuclear and cytoplasmic features of hepatocytes were normal. There was no increase in peroxisome size or number.

Liver Enzymes

The thermogenic enzymes, mitochondrial glycerophosphate dehydrogenase and cytosolic malic enzyme, are induced in rat liver by thyroid hormone and by dehydroepiandrosterone and its 7-keto derivative. The small increases in hepatic glycerophosphate dehydrogenase following the 140 mg/kg 7-keto DHEA feeding period were not significant. Malic enzyme did increase somewhat as a result of feeding with either 7-keto-DHEA or thyroxine.

Discussion

Feeding DHEA and its 7-oxygenated derivatives to rats increases liver size as a result of increased peroxisome formation. No such alterations were found in the livers of monkeys fed as much as 70 mg/kg body wt of 7-keto-DHEA for 14 days. Body weights were not detectably affected by treating with 7-keto-DHEA. These findings, together with experiments in rats showing no toxicity from single doses of 2000 mg/kg indicates that 7-keto-DHEA is well tolerated.

Acknowledgments

This work was supported by grants AG-07831 and RR-00167 from the National Institutes of Health and by Humanetics Corp., St. Louis Park, MN. The protocol for this project was approved by the Graduate School Animal Care and Use Committee of the University of Wisconsin. The Wisconsin Regional Primate Research Center (WRPRC) is fully accredited by American Association of Accreditation of Laboratory Animal Care.

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